U.S. ENVIRONMENTAL PROTECTION AGENCY
REGION 10
1200 SIXTH AVENUE
SEATTLE, WASHINGTON 98101

File: WA-000190-2

MAY 19 1989

REPLY TO
ATTN OF: WD-134

James W. Warren
U.S. Fish and Wildlife Service
Lloyd 500 Building, Suite 1692
500 N.E. Multnomah Street
Portland, Oregon 97232

Re: Discharge of Malachite Green

Dear Mr. Warren:

This is in response to your March 13, 1989, letter requesting an interim or temporary permit for the use of malachite green in the treatment of fish and eggs in Pacific Northwest hatcheries. Your letter identifies hatcheries using malachite green in Idaho, Washington, Oregon and other areas of the United States. Our response addresses only those hatcheries that are permitted by the Environmental Protection Agency (EPA). See Enclosure 1 for a list of those facilities. For information on hatcheries in Washington and Oregon, I recommend you contact the Washington Department of Ecology and Oregon Department of Environmental Quality since those state agencies have been delegated permitting authority under the National Pollutant Discharge Elimination System (NPDES).

Based on a review of the permit files for the Idaho facilities and the federal hatcheries in Washington, together with the information you have provided, we have determined that it is not necessary to obtain an interim or temporary permit in order to use malachite green. The use of malachite green and other disease controlling drugs has long been recognized as necessary at fish rearing facilities. The permits for the Idaho facilities contain a prohibition on the discharge of such drugs in toxic amounts. The Washington federal hatchery permits contain no conditions specifically addressing drug uses. Nonetheless, the prohibition on discharges in toxic amounts still apply.

We have reviewed the available literature regarding the toxicity of malachite green and have concluded that the discharge of malachite green to waters of the United States at levels less than 0.1 mg/l would not result in long term toxic effects.

The NPDES permits for the Idaho hatcheries will expire in October 1989. The Washington federal hatchery permits have expired. The permit reissuance process for these facilities will begin this summer. The new permits may include effluent limits and/or best management practices for applying malachite green and other drugs or chemicals in hatchery waters.

Questions on this issue may be referred to Roger Mochnick at (206) 442-4817.

Sincerely,

Harold Geren

Harold E. Geren, Chief
Water Permits and Compliance Branch

Enclosures

Questions on this issue may be referred to Roger Mochnick at (206) 442-4817.

Sincerely,

/s/ Harold E. Geren

Harold E. Geren, Chief
Water Permits and Compliance Branch

Enclosures

bcc: IOO
WOO
OOO
WCS
copy for each NPDES facility listed in Enclosure 1

	CONCURRENCES							
SYMBOL	Kawabata	5-9-89	1254N					
SURNAME	KAWABATA	MOCHNICK						
DATE								

EPA Form 1320-1 (12-70)

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★U.S.GPO:1988-0-206-471

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CONCURRENCES

SYMBOL								
SURNAME								
DATE								

★U.S.GPO:1988-0-206-471

ENCLOSURE 1

Idaho Facilities

Idaho Power Co.
Pahsimeroi Hatchery
NPDES No. ID-002252-7

Idaho Power Co.
Rapid River Hatchery
NPDES No. ID-002237-3

Idaho Fish and Game
McCall Hatchery
NPDES No. ID-002508-9

Idaho Fish and Game
Sawtooth Hatchery
NPDES No. ID-002644-1

Dept. of Interior
U.S. Fish and Wildlife
Dworshak National Fish Hatchery
NPDES No. ID-002151-2

Dept. of Interior
U.S. Fish and Wildlife
Kooskia National Fish Hatchery
NPDES No. ID-000081-7

Washington Facilities

Dept. of Interior
U.S. Fish and Wildlife
Carson National Fish Hatchery
NPDES No. WA-000020-5

Dept. of Interior
U.S. Fish and Wildlife
Entiat National Fish Hatchery
NPDES No. WA-000188-1

Dept. of Interior
U.S. Fish and Wildlife
Leavenworth National Fish Hatchery
NPDES No. WA-000190-2

Dept. of Interior
U.S. Fish and Wildlife
Little White Salmon National
Fish Hatchery
NPDES No. WA-000021-3

Dept. of Interior
U.S. Fish and Wildlife
Quilcene National Fish Hatchery
NPDES No. WA-000187-2

Dept. of Interior
U.S. Fish and Wildlife
Winthrop National Fish Hatchery
NPDES No. WA-000259-3

References

Bills, Terry D., Marking, Leif L., and Chandler J.H., Jr. (1977) "Malachite green: Its Toxicity to Aquatic Organisms, Persistence and Removal with Activated Carbon," Investigations in Fish Control 75, 6 pp.

Little, Linda W., Lamb III, James C., Chillingworth, Mary Anne, Durkin, William B., "Acute Toxicity of Selected Commercial Dyes to the Fathead Minnow and Evaluation of Biological Treatment for Reduction of Toxicity," Proceedings of the 29th Industrial Waste Conference May 7, 8 and 9, 1974, pp 524 - 534.

Wilford, Wayne A., (1966), "Toxicity of 22 Therapeutic Compounds to Six Fishes," Investigation in Fish Control 18, 10 pp.

from: Investigations in Fish Control, 75 (1977)

Malachite Green: Its Toxicity to Aquatic Organisms, Persistence and Removal with Activated Carbon

by

Terry D. Bills and Leif L. Marking
Fish Control Laboratory, La Crosse, Wisconsin

Jack H. Chandler, Jr.
Southeastern Fish Control Laboratory
Warm Springs, Georgia

Abstract

The acute toxicity of malachite green was determined in standardized laboratory tests for chinook salmon *(Oncorhynchus tshawytscha)*, coho salmon *(O. kisutch)*, Atlantic salmon *(Salmo salar)*, brown trout *(S. trutta)*, rainbow trout *(S. gairdneri)*, brook trout *(Salvelinus fontinalis)*, channel catfish *(Ictalurus punctatus)*, largemouth bass *(Micropterus salmoides)*, smallmouth bass *(M. dolomieui)*, bluegill *(Lepomis macrochirus)*, snails (*Pleurocera* sp.), Asiatic clams *(Corbicula leana)*, ostracods (*Cypridopsis* sp.), freshwater prawns *(Palaemonetes kadiakensis)*, larval midges *(Tanytarsus dissimilis)*, naiads of mayflies (*Callibaetis* sp.), adult newts *(Notophthalmus viridescens)*, larval leopard frogs *(Rana pipiens)*, and larval toads (*Bufo* sp.). Bluegills were the most sensitive (96-h LC_{50}, 0.0305 mg/l), and coho salmon the most resistant (0.383 mg/l). The $TILC_{50}$ (lethal concentration producing 50% mortality independent of time) for rainbow trout was 0.0998 mg/l. The responses of frog and toad larvae (96-h LC_{50}, 0.173 and 0.0680 mg/l) were similar to those of fish, whereas adult newts were more resistant (1.03 mg/l). The invertebrates exposed were generally more resistant than the fish and amphibians; the 96-h LC_{50}'s ranged from 0.510 to 3.45 mg/l, except for the Asiatic clam, which was extremely resistant (122 mg/l), and the mayfly naiad, which was very sensitive (0.0790 mg/l). The toxicity of malachite green to fish was not affected by water hardness or pH, except bluegills, in which toxicity was about half as great at pH 6.5 as at pH 7.5 to 9.5, and was increased only slightly by increases in water temperatures. Malachite green was very persistent in aqueous solutions; it did not detoxify after 3 weeks of aging in glass containers. The chemical is readily absorbed from aqueous solutions (pH 7.5, total hardness 44 mg/l, temperature 12 C) by filtration through activated carbon; the capacity was 23.4 mg of malachite green per gram of carbon.

Malachite green has been used in fish culture as a fungicide and parasiticide for about 40 years. It was first used as a dip treatment by Foster and Woodbury (1936) to treat fungal infections of four species of trout and largemouth bass *(Micropterus salmoides)*. More recently it has been used in combination with formalin to treat *Ichthyophthirius*, a serious parasite of catfishes (Leteux and Meyer 1972).

Although the use of malachite green as a therapeutant in fish culture has many advantages, it also poses various potential problems (Nelson 1974): toxicity to fishes (Willford 1967); possible teratogenic and mutagenic effects (Lieder 1961; Nelson 1974; T.D. Bills and L.L. Marking, in preparation); and stress induced during and after the treatment of fry of certain fishes (Glagoleva and Malikova 1968; Bills and Hunn 1976).

Malachite green is not registered for aquatic use by either the Food and Drug Administration or Environmental Protection Agency, because information required for registration—toxicity, efficacy, residues, metabolites, and counteraction—is incomplete. The purpose of the present study was to contribute laboratory data on (1) the toxicity of malachite green to nontarget aquatic organisms; (2) its toxicity to rainbow trout *(Salmo gairdneri)* and bluegills *(Lepomis macrochirus)* in extended exposures; (3) the effects of certain water characteristics on its toxicity to fish; (4) its persistence in water; and (5) its possible removal from water with activated carbon.

Materials and Methods

Concentrated stock solutions of commercial grade zinc-free malachite green (4-[P-(dimethylamino)-α-phenylbenzylidene]-2,5-cyclohexadien-1-ylidene dimethyl-ammonium chloride) manufactured by MCB Manufacturing Chemists, Norwood, Ohio, were prepared by mixing weighed portions with water. To prepare test solutions of the desired concentrations, we pipetted portions of stock solutions into test vessels and stirred the resulting mixture to ensure homogeneity. In flow-through toxicity tests, the required amounts of the stock solution were delivered by a solenoid-activated pipette pump (Micromedic Systems Automatic Pipette Model 2500).

Tests were conducted according to the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and the protocol described by Marking (1975). Glass jars of 3.78 or 18.9 liters were used, depending on the size of the test organism. Reconstituted water was used in tests with fish (Marking 1969), and limed spring water (pH, 7.5 ± 0.1; total hardness, 20 mg/l as $CaCO_3$) in the tests with amphibians and invertebrates. Chemical buffers were added to soft water to adjust the pH (6.5–9.5), as described by Marking and Dawson (1973).

Flow-through tests were conducted in a proportional diluter similar to that of Mount and Brungs (1967). Test vessels were 45-liter glass aquariums supplied with a flow sufficient to replace the entire volume at least four times daily. Carbon-filtered, municipal well water (total hardness 300 mg/l, pH 7.5) was used in the flow-through system. Temperature was maintained by immersing test vessels in a water bath equipped with a chilling unit.

Fish species exposed were chinook salmon *(Oncorhynchus tshawytscha)*, coho salmon *(O. kisutch)*, Atlantic salmon *(Salmo salar)*, brown trout *(S. trutta)*, rainbow trout, brook trout *(Salvelinus fontinalis)*, channel catfish *(Ictalurus punctatus)*, largemouth bass, smallmouth bass *(Micropterus dolomieui)*, and bluegills. Test fishes weighed 0.5 to 1.5 g. Other aquatic organisms exposed were snails (*Pleurocera* sp.), Asiatic clams *(Corbicula leana)*, ostracods (*Cypridopsis* sp.), freshwater prawns *(Palaemonetes kadiakensis)*, larval midges *(Tanytarsus dissimilis)*, naiads of mayflies (*Callibaetis* sp.), adult newts *(Notophthalmus viridescens)*, larval leopard frogs *(Rana pipiens)*, and larval toads (*Bufo* sp.).

In tests for the determination of persistence of malachite green, aqueous solutions were aged for 1, 2, and 3 weeks in glass containers. Rainbow trout were introduced concurrently to these and a freshly prepared reference solution for comparison of mortality. Deactivation indices were computed from these data according to the method of Marking (1972).

We used the method of Litchfield and Wilcoxon (1949) to determine LC_{50}'s and 95% confidence intervals, and a modification of the method published by Green (1965) for computing TILC$_{50}$'s (lethal) concentration producing 50% mortality independent of time).

To determine if malachite green could be removed from aqueous solutions (pH 7.5, total hardness 44 mg/l, temperature 12 C), we filtered a concentrated solution (2 mg/l) at a flow rate of 100 ml/min through a glass 2.7 cm ID column containing 15 cm (85.5 g dry weight) of activated carbon (Darco 20 × 40 mesh). Samples were taken periodically and concentrations in the effluent determined colorimetrically (620 nm). The carbon bed was considered saturated when the concentration in the effluent reached 10% of that in the original stock solution (0.2 mg/l). The capacity of activated carbon for the chemical was determined by the following formula:

$$\text{Milligrams of malachite green adsorbed per gram of carbon} = \frac{\text{Concentration (mg/l)} \times \text{liters passed through filter}}{\text{Grams of carbon (dry weight)}}$$

Results

Toxicity to Fish

Malachite green was toxic to all species of fish exposed; LC_{50}'s ranged from 0.0305 to 0.383 mg/l in 96-h exposures in soft water at 12 C (Table 1). Centrarchids were 1.5 to 3.5 times more sensitive to the chemical than the ictalurids and 3 to 7 times more sensitive than the salmonids. The bluegill was the most sensitive species (96-h LC_{50}, 0.0305 mg/l) and the coho salmon the most resistant (0.383 mg/l). The toxicity of the chemical increased as exposures lengthened in all species; for bluegills the LC_{50} was 6.00 mg/l at 3 h and 0.0305 mg/l at 96 h.

Toxicity to Other Aquatic Organisms

In 96-h exposures, the LC_{50}'s for malachite green to frog larvae (0.173 mg/l) and toad larvae (0.0680 mg/l) were similar to those for fish (Table 2). Adult newts were more resistant than frog or toad larvae (96-h LC_{50}, 1.03 mg/l), but about equally or less resistant than most of the invertebrates exposed. Mayfly naiads were the most sensitive invertebrate

Table 1. *Toxicity of malachite green to fingerling fish in soft water at 12 C.*

Species	LC_{50} and 95% confidence interval (mg/l) at			
	3 h	6 h	24 h	96 h
Chinook salmon *(Oncorhynchus tshawytscha)*	1.72 1.22–2.42	1.38 1.04–1.82	0.292 0.245–0.348	0.224 0.209–0.240
Coho salmon *(O. kisutch)*	— —	>3.00	0.569 0.486–0.662	0.383 0.327–0.449
Atlantic salmon *(Salmo salar)*	3.56 2.77–4.58	1.09 0.929–1.28	0.497 0.415–0.595	0.283 0.229–0.350
Brown trout *(S. trutta)*	1.73 1.23–2.43	1.27 0.991–1.63	0.352 0.280–0.443	0.237 0.209–0.268
Rainbow trout *(S. gairdneri)*	1.41 1.14–1.74	0.760 0.649–0.890	0.360 0.305–0.425	0.248 0.193–0.319
Brook trout *(Salvelinus fontinalis)*	3.00 2.06–4.37	1.44 1.05–1.98	0.300 0.259–0.348	0.220 0.188–0.257
Channel catfish *(Ictalurus punctatus)*	>3.00	1.10 0.904–1.34	0.181 0.123–0.266	0.112 0.0893–0.140
Largemouth bass *(Micropterus salmoides)*	— —	— —	0.282 0.211–0.376	0.0728 0.0604–0.0877
Smallmouth bass *(M. dolomieui)*	1.36 1.09–1.70	— —	0.154 0.117–0.202	0.0453 0.0366–0.0561
Bluegill *(Lepomis macrochirus)*	6.00 4.41–8.17	2.19 1.66–2.89	0.237 0.184–0.290	0.0305 0.0218–0.0427

Table 2. *Toxicity of malachite green to selected nontarget aquatic organisms in limed water at 16 C.*

Organism	LC_{50} and 95% confidence interval (mg/l) at		
	6 h	24 h	96 h
Snail *(Pleurocera* sp.*)*	— —	— —	0.720 0.483–1.07
Asiatic clam *(Corbicula leana)*	— —	— —	122 93.8–159
Ostracod *(Cypridopsis* sp.*)*	5.85 4.00–8.57	5.85 4.29–7.97	3.45 2.49–4.80
Freshwater prawn *(Palaemonetes kadiakensis)*	— —	9.10 7.29–11.3	1.90 1.76–2.06
Midge (larvae) *(Tanytarsus dissimilis)*	5.00 3.13–7.99	1.00 0.636–1.57	0.510 0.295–1.10
Mayfly naiads *(Callibaetis* sp.*)*	5.75 4.95–6.69	2.75 2.07–3.65	0.0790 0.0442–0.141
Newts (adult) *(Notophthalmus viridiscens)*	— —	3.90 3.47–4.38	1.03 0.672–1.58
Leopard frog (larvae) *(Rana pipiens)*	1.00 0.875–1.14	0.380 0.351–0.412	0.173 0.149–0.200
Toad (larvae) *(Bufo* sp.*)*	1.70 1.54–1.87	0.355 0.235–0.276	0.0680 0.0530–0.0860

Table 4. *Toxicity of malachite green to fingerling channel catfish at selected temperatures, water hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l at 6 h	24 h	96 h
12	Soft	7.5	1.10 0.904–1.34	0.181 0.123–0.266	0.112 0.0893–0.140
17	Soft	7.5	0.552 0.499–0.610	0.222 0.168–0.293	0.0940 0.0860–0.103
22	Soft	7.5	0.400 0.331–0.483	0.0691 0.0576–0.0831	0.0535 0.0442–0.0647
12	Very soft	8.0	0.600 0.440–0.818	0.106 0.0935–0.120	0.0750 0.0555–0.101
12	Soft	8.0	1.30 1.01–1.67	0.285 0.232–0.350	0.117 0.0972–0.140
12	Hard	8.0	1.72 1.23–2.41	0.284 0.229–0.351	0.142 0.115–0.176
12	Very hard	8.0	1.71 1.22–2.40	0.286 0.232–0.353	0.142 0.115–0.176
12	Soft	6.5	0.960 0.717–1.29	0.236 0.181–0.308	0.0975 0.0937–0.101
12	Soft	8.5	0.835 0.665–1.05	0.835 0.665–1.05	0.237 0.182–0.309
12	Soft	9.5	0.519 0.377–0.714	0.191 0.155–0.236	0.162 0.135–0.194

Table 5. *Toxicity of malachite green to fingerling bluegill at selected temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l) at 3 h	6 h	24 h	96 h
12	Soft	7.5	6.00 4.41–8.17	2.19 1.66–2.89	0.231 0.184–0.290	0.0305 0.0218–0.0427
17	Soft	7.5	2.17 1.63–2.88	0.656 0.584–0.737	0.0920 0.0663–0.128	0.0340 0.0242–0.0477
22	Soft	7.5	0.860 0.737–1.00	0.238 0.184–0.308	0.0780 0.0594–0.102	0.0308 0.0221–0.0430
12	Very soft	8.0	2.30 1.72–3.08	2.00 1.54–2.59	0.117 0.0967–0.142	0.0413 0.0343–0.0497
12	Soft	8.0	>2.00	1.52 1.15–2.00	0.122 0.100–0.149	0.0400 0.0330–0.0486
12	Hard	8.0	>2.00	1.41 1.14–1.74	0.141 0.114–0.174	0.0450 0.0384–0.0528
12	Very hard	8.0	>2.00	1.42 1.10–1.83	0.141 0.114–0.174	0.0440 0.0370–0.0523
12	Soft	6.5	7.43 5.76–9.59	2.18 1.64–2.90	0.282 0.219–0.394	0.0780 0.0594–0.102
12	Soft	8.5	4.68 3.77–5.80	2.18 1.64–2.89	0.123 0.0955–0.158	0.0339 0.0241–0.0476
12	Soft	9.5	3.70 2.81–4.87	2.20 1.67–2.89	0.0810 0.0562–0.117	0.0340 0.0242–0.0477

Counteraction with Activated Carbon

Aqueous solutions of malachite green (2.0 mg/l) were filtered through a bed of activated carbon. In three runs the activated carbon adsorbed the chemical from 420, 401, and 425 liters of solution before the endpoint was reached (0.2 mg/l), an average of 23.4 mg of malachite green per gram of carbon. Activated carbon thus is an excellent means for removing this chemical from water.

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From Proceedings 29th Industrial Waste Conference Purdue Univ, 1974

Acute Toxicity of Selected Commercial Dyes to the Fathead Minnow and Evaluation of Biological Treatment for Reduction of Toxicity

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INTRODUCTION

Man's use of dyes dates back to the Stone Age or earlier, and by 3000 B.C. the dyeing industry was well developed in Egypt, Mesopotamia, and India. The first synthesis of an organic dye was achieved in 1771 when Woulfe produced picric acid by treating the natural dye indigo with nitric acid. The first man-made organic dye was mauve, produced by Perkin in 1856. Since the 1850's, hundreds of new synthetic dyes have been added to the dyer's repertoire.

Despite the long history of dyes and dyeing, little is known of the effects of dyes on living organisms. In 1970, the American Dye Manufacturer's Institute, Inc. initiated research to evaluate the toxicity of selected dyes on selected organisms representative of receiving stream biota. Criteria for selection of dyes included chemical structure and production volume, and an effort was made to include dyes representing most of the important chemical and application classes (1). In June, 1971, the UNC Wastewater Research Center began a study of the toxicity to fish and algae of the 46 dyes chosen. This report presents results of acute toxicity bioassay studies with the fathead minnow, *Pemephales promelas*. For those dyes showing marked toxicity to fish, aerobic biological treatment is being evaluated as a means of reducing color and toxicity. Preliminary results of these studies are described.

MATERIALS AND METHODS

The testing procedure adhered closely to the static bioassay described in *Standard Methods for the Examination of Water and Wastewater* (2). That procedure permits considerable flexibility and specific materials and methods employed in these tests are presented in this paper.

The static bioassay method to evaluate toxicity has been criticized because it relates only to acute toxicity (i.e., death) and does not take into account many other aspects; more complex alternate methods have been suggested (2). Nevertheless, this type of bioassay has been, and will probably continue to be, widely used because of its relative simplicity and economy.

A major objection to reported fish bioassay results in general is that seldom has sufficient information been published on the test organism, dilution water and test conditions (3, 4). A special effort was made to avoid this criticism by preparing a data sheet

designed to include all pertinent information for each test. Data sheets for each dye tested are available in an ADMI report (5).

The fathead minnow, *Pimephales promelas,* was selected from a list of recommended species prepared by Dr. D.I. Mount of the National Water Quality Laboratory (3). This species has been widely used in fish bioassay studies, proved adaptable to laboratory conditions, and was readily available locally. Test fish were obtained from Berry Water Gardens and from Windmill Fish Hatcheries, both located in Kernersville, North Carolina.

New shipments of fish were routinely exposed on arrival to the broad-spectrum antibiotic tetracycline HCl (Tetrachel®, Rachelle Laboratories, Inc., Long Beach, California) at a dose of 50 mg per gallon of water for 24-48 hours. Such treatment is necessary to prevent introduction into the stock tank of diseases from fishery stock or from fish damaged in shipment (6). Upon any evidence of disease in the stock tanks, the tetracycline treatment was repeated.

Fish used in full-scale bioassay tests were maintained in a 250-gallon polyethlyene tank equipped with aeration devices. Water was renewed continuously by introduction of fresh tap water from the City of Chapel Hill, pretreated to remove chlorine and organic carbon (see next section). A thermostatic device prevented the temperature from dropping below 14 C.

Fish were acclimatized to test temperature and the experimental dilution water for a minimum of ten days before testing. They were fed 5-7 times per week with protein-enriched commercial fish food. For 48 hours prior to testing, the fish were starved, as customary in static tests to reduce the amount of waste materials generated by them in the test container.

Experimental Dilution Water

The purpose of the bioassays was not to evaluate the effect of dye waste discharges on fish native to some specific receiving stream, but, instead, to evaluate the effects of a large number of dyes on one fish species. Since reproducibility and general application of the results were desired, experimental dilution water of constant quality was essential. To obtain such water, Chapel Hill tap water was dechlorinated and filtered by passage through an activated carbon-sand filter system (Carbo-Dur and coarse sand system, Permutit Water Conditioning, Inc.). The water was analyzed according to *Standard Methods* (2) before each test series. Its average chemical and physical characteristics are shown in Table I.

TABLE I

CHARACTERISTICS OF EXPERIMENTAL DILUTION WATER

Parameter	Range	Median
pH	6.6-7.1	6.8
Total Organic carbon, mg/l	2-34	4
Fe, mg/l	<0.01-0.63	<0.02
Al, mg/l	<1.0	<1.0
Mg, mg/l	0.53-1.6	1.4
Ca, mg/l	5.1-8.7	8.5
Total dissolved solids, mg/l	85-138	121
Turbidity, JTU	0-2	0
Total Alkalinity as $CaCO_3$, mg/l	19-32	27

Dyes

Dyes tested were supplied by ADMI and represented composites from a number of manufacturers (see Appendix). The color index number and name of each dye is shown in Table II. Current commercial names are available from the annual AATCC publication Products/73 or from the Colour Index (7). Vat dyes were supplied as dispersions in Tamol

SN® (Rohm and Hass, Co.); disperse dyes, as dispersions in Reax 85A® (Westvaco). Other dyes were supplied as dry powders.

Dyes supplied as liquids were initially stored at about 20 C and subsequently refrigerated. Dry powders were stored in the dark at about 20 C. All were stored in the containers in which they were received.

Concentrations of dyes are expressed in terms of milligrams per liter of the dye. For dyes supplied as dry powders, the weight is that of the powder; no attempt was made to determine the concentration of trace and inert materials. For dyes supplied as liquid solutions or suspensions, the weight of the dye was calculated from information supplied by ADMI. For example, for a 15% (by weight) dispersion of Disperse Yellow in 15% Reax, the weight is calculated on the basis of the 15% dye.

$$\text{mg/l dye} = \text{mg/l of 15\% solution added} \times 0.15$$

For both liquid and dry dye preparations, test solutions were prepared by weighing out the appropriate dye on an analytical balance (Mettler P1200 or H20T).

Temperature for Tests

Temperature is known to affect response of fish to toxic materials (8, 9, 10, 11). The testing laboratory was equipped with air conditioning, thermostatically-controlled heaters, and circulating fans in order to maintain constant (± 2 C) temperature. Although Average water temperature in the various test series varied from 15 to 20 C, within any given test the temperature range did not exceed ± 2 C, as specified in *Standard Methods* (2). Temperature was measured at the beginning, middle, and end of the 96-hour exposure period with a thermistor probe (Yellow Springs Instrument Co.) or a common laboratory thermometer. Ambient temperature in the laboratory was monitored continuously with a recording thermometer (Tempscribe).

Dissolved Oxygen Content and Aeration of Test Solutions

Dissolved oxygen (DO) is required by fish for survival. Also, DO is a factor in response of fish to toxic materials (10, 11). *Standard Methods* (2) states that the DO should not drop below 5 mg/l in fish bioassay tests. However, fish can tolerate oxygen concentrations well below 3 mg/l, especially when maintained under laboratory conditions with restricted feeding (12).

Aeration of test solutions during static fish bioassay tests is not recommended if there is a possibility that such aeration may affect toxicity by accelerating loss of volatile materials. In order to avoid loss of components and yet maintain a safe dissolved oxygen level the following steps were taken: 1) No feeding of fish was allowed during the test period; 2) Fish were starved for 48 hours prior to testing; 3) The experimental dilution water was allowed to equilibrate with the atmosphere prior to testing; 4) Dead fish were removed as soon as observed; and 5) Aeration with compressed air was employed for only 5 minutes per jar after 48 hours of testing.

Dissolved oxygen concentration was measured initially and at 48-hour intervals during tests, using a polarographic oxygen analyzer (Yellow Springs Instrument Company).

pH Determination

pH was determined at the beginning, middle and end of the 96-hour exposure with a Leeds & Northrup pH meter equipped with combination probe and expanded scale. No attempt was made to control pH during these tests; however, little variation in pH was noted.

Test Concentrations and Procedures

Small-scale exploratory bioassays were conducted to determine the range of concentrations to be tested in full-scale tests. For small-scale tests, solutions were prepared with the following concentrations: 0.01, 0.1, 1.0, 10, and 100 mg/l. A test volume of 3.5 liters and 2-3 fish per container were used.

TABLE II
TOXICITY OF SELECTED DYES TO THE FATHEAD MINNOW, *PIMEPHALES PROMELAS*, AND COMPARISON OF TL_{50} TO CONCENTRATIONS PRODUCING COLOR

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Dye Class	CI #	Dye	TL_{50}, mg/l			Temp C	Concentration, mg/l to produce color		
			24 hr	48 hr	96 hr		50 APHA	100 APHA	150 APHA
Nitro	10338	Disperse Yellow 42	>180	>180	>180	15	7.8	16.5	25.2
Monoazo	11855	Disperse Yellow 3	>180	>180	>180	15	2.9	6.1	9.3
	14645	Mordant Black 11	10.0	6.0	6.0	15	0.3	0.9	1.4
	15510	Acid Orange 7	>180	>180	165	17	0.3	0.7	1.0
	15711	Acid Black 52	7.0	6.2	6.2	15	1.2	2.3	3.4
	18965	Acid Yellow 17	>180	>180	>180	17	0.7	1.4	2.2
	19555	Direct Yellow 28	>180	180	>180	15	0.8	1.5	2.2
		Acid Yellow 151	29	29	29	15	0.6	1.0	1.5
Diazo	20170	Acid Orange 24	>180	>180	130	17	0.2	0.5	0.7
	20470	Acid Black 1	>180	>180	>180	15	0.3	0.6	0.9
	21010	Basic Brown 4	> 10	7.5	5.6	20	0.35	0.7	1.1
	22610	Direct Blue 6	>180	>180	>180	17	0.4	0.9	1.3
	24401	Direct Blue 218	>180	>180	>180	17	0.4	0.8	1.4
	24890	Direct Yellow 4	>180	>180	>180	15	0.2	0.4	0.7
	24895	Direct Yellow 12	>180	130	125	15	0.3	0.6	1.1
	25135	Acid Yellow 38	24	24	23	15	0.9	2.0	3.3
	26360	Acid Blue 113	4.5	4.5	4.4	15	0.4	0.8	1.2
	28160	Direct Red 81	>180	>180	>180	17	0.3	0.5	0.8
	29025	Direct Yellow 50	>180	>180	>180	17	0.6	1.5	2.3
	29160	Direct 23	>180	>180	>180	17	0.6	1.2	1.8
	30145	Direct Brown 95	>180	>180	>180	17	0.4	0.7	1.1
Polyazo	30235	Direct Black 38	>180	>180	>180	17	0.4	0.9	1.5
	31600	Direct Black 80	>180	>180	>180	17	0.2	0.3	0.5
	40000	Direct Yellow 11	>180	>180	>180	17	0.4	0.8	1.3
Stilbene	40622	Fluroescent Brightening Agent 28	>180	>180	>180	17	--	--	--
		Direct Yellow 106	>180	>180	>180	17	0.5	1.0	1.9
Di- and Tri-phenylmethane	42000	Basic Green 4	> 0.2	0.12	0.12	18	0.1	0.3	0.4

Dye Class	CI #	Dye	TL_{50}, mg/l			Temp C	Concentration, mg/l to produce color	
			24 hr	48 hr	96 hr		50 APHA	100 APHA 150 APHA
Quinoline	42535	Basic Violet 1	0.21	0.13	0.047	15	0.1	0.2 0.3
Polymethane	47020	Disperse Yellow 54	>180	>180	>180	15	4.9	9.9 14.9
Oxazin	48055	Basic Yellow 11	4.4	4.0	3.2	18	0.3	0.6 0.9
Thiazin	51005	Basic Blue 3			4.0	15	0.1	0.3 0.4
	53185	Sulfur Black 1	>180	>180	>180	15	7.8	16 23
Anthraquinone	53630	Vat Blue 43	>180	>180	>180	15	3.2	6.4 9.5
	59105	Vat Orange 1	>180	>180	>180	15	5.2	12.2 19.2
	59825	Vat Green 1	>180	>180	>180	15	6.7	13.4 20.0
	61505	Disperse Blue 3	> 1	> 1	1	15	2.0	4.0 6.1
	61570	Acid Green 25	> 10	5.7	6.2	18	0.9	1.8 2.7
	62055	Acid Blue 25	12.5	12.5	12	15	0.5	1.0 1.5
	62500	Disperse Blue 7	>180	142	52	15	2.5	5.0 7.5
	63010	Acid Blue 45	>180	>180	>180	15	0.5	1.0 1.5
	67300	Vat Yellow 2	>180	>180	>180	>180	15	
	69015	Vat Brown 3	>180	>180	>180	15	9.5	18.5 28.3
	69500	Vat Green 3	>180	>180	>180	15	16.5	34.5 57
	69825	Vat Blue 6	>180	>180	>180	15	8.5	18 28
		Disperse Red 60	>180	>180	>180	15	3.1	6.2 9.4
Phthalocyanine	74180	Direct Blue 86	>180	>180	>180	17	0.6	1.3 2.0

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Based on results of small-scale bioassays a full-scale test range was chosen, the concentrations falling between the highest concentration at which all fish survived and the lowest concentration at which all or most of the fish died. An exception was made for dyes in which all fish survived at 100 mg/l. In such cases 180 mg/l was highest concentration tested in the full-scale tests. It was assumed that higher concentrations were unrealistic and not likely to be encountered in streams receiving dye wastes.

In full-scale tests the TL_{50} was determined by testing a series of five concentrations chosen from *Standard Methods* (2) and based on progressive bisection of intervals on the logarithmic scale, i.e., 1.0, 1.8, 3.2, 5.6, and 10.0 mg l, multiplied or divided as necessary by any power of 10.

In each test series, control tests were conducted concurrently with the experimental dilution water alone. No more than 10% mortality occurred among control fish during the tests.

In most instances, 10 fish were used to test each experimental concentration. In some cases the number varied from 9 to 20 because of difficulties incurred by reduced visibility in solutions of high dye concentration.

Test containers were 5-gallon wide-mouth glass jars (Smith Container Corporation, Charlotte, N.C.), 25 cm (d) x 47 cm (h) and contained 15 liters of test solution. Prior to use, they were washed thoroughly with a cleaning solution (Micro, International Products Corporation) found to be effective in removing dyes. After washing, they were rinsed thoroughly with tap water, with acetone (if necessary), and finally with distilled water.

Test Duration and Observations

Duration of all tests was 96 hours; fish were observed at 24-hour intervals. As soon as observed, dead fish were removed, weighed, and measured. At the end of the test, remaining fish were weighed and measured. Weighing and measuring of fish after, rather than before the test, reduced excessive handling which could have damaged or made them more susceptible to toxic compounds.

RESULTS AND DISCUSSION

Effects of the 46 dyes on fathead minnows were determined in a 96-hour static bioassays at concentrations up to 180 mg/l. The TL_{50}, the concentration at which 50% of the experimental animals survive, was estimated by interpolation after plotting percentages to fish surviving at each concentration on semilogarithmic coordinate paper.

A complete listing of the data is shown in Table II. The TL_{50} values for 29 dyes were higher than 180 mg/l; 3 others fell between 100 and 180 mg/l; and the remaining 14 were lower than 100 mg/l. The most toxic dyes were Basic Violet 1 (methyl violet), with a TL_{50} of 0.047 mg/l; Basic Green 4 (malachite green), 0.12 mg/l; Disperse Blue 3, 1 mg/l; Basic Yellow 11, 3.2 mg/l; Basic Blue 3, 4 mg/l; Acid Blue 113, 4 mg/l; Basic Brown 4, 5.6 mg/l; Mordant Black 11, 6 mg/l; Acid Green 25, 6.2 mg/l; and Acid Black 52, 7 mg/l. In addition, Table II presents the dye concentrations producing color of 50, 100, and 150 APHA units.

The two most toxic compounds, Basic Violet 1 and Basic Green 4, are triphenylmethane dyes. Malachite green (Basic Green 4) long has been used as a therapeutic fungicidal compound for fish (6, 13, 14). It is an effective drug at appropriate concentrations, but is known to be toxic at higher concentrations. Willford (14) tested 22 therapeutic compounds using six fishes (rainbow trout, brown trout, brook trout, lake trout, bluegills, and channel catfish) and found malachite green to be most toxic, with a TL_{50} of 0.1-0.4 mg/l. He concluded that there was risk associated with long-term treatment with malachite green at concentrations in excess of 0.11 mg/l. Lanzing (15) stated that there was not significant difference in the oxalate and chloride forms of malachite green in regard

to toxicity to whiting. In a recent survey of literature on effects of chemicals in aquatic life, numerous instances of malachite green toxicity to fish are cited, including rainbow trout, 2-5 day TL_{50} = 0.122 mg/l, 18 day TL_{50} = 0.048 mg/l, channel catfish, 2 day TL_{50} = 0.14 mg/l (4).

Malachite green has also been used as a bacteriostatic and amebicidic agent (16). Indeed, the triphenylmethane dyes, as a group, affect the viability of bacteria (17, 18, 19) and, depending on concentration, may be bacteriostatic or bactericidal.

The antibacterial activity of triphenylmethane dyes is attributed to their cationic nature. It is suggested that the activity is due to "a reaction of the cation with some anionic groups of bacteria to give fully dissociated complexes" (17). Albert (17) and co-workers showed that a quantitative relationship exists between antibacterial action and ionization as cations in the acridine series. He also cites work by Goldacre and Phillips indicating strong correlation between ionization and antibacterial activity of the triphenylmethane dyes, Doebner's violet, malachite green, and brilliant green. He points out the dependence of ionization, and thus of antibacterial activity, on presence of chemically inert groups in the dye molecule.

Fung and Miller (7) tested the effect of 42 dyes on growth of 30 bacterial species on solid media. Dyes were tested at 1:1,000 dilution. The basic aminotriphenylmethane dyes brilliant green and malachite green inhibited growth, respectively, of 26 and 10 species. The basic triaminotriphenylmethane dyes were inhibitory as follows: crystal violet, 27 species; basic fuchsin, 16; p-rosaniline, 19 methyl green, 18; methyl violet, 24. Bismark brown (Basic Brown 4, a diazo dye), was inhibitory to 4. Fung and Miller indicated that gram-negative species are more resistant to dyes than are gram positive species, and that at the same concentration, basic dyes are more inhibitory than acid and neutral dyes.

No information has been found in the literature concerning the effect of Basic Violet 1 on fish. However, gentian violet, a similar dye, is used as a therapeutic agent for fish (6).

The biological effects of Disperse Blue 3, Basic Yellow 11, Basic Blue 3, Acid Blue 113, Mordant Black 11, Acid Green 25, and Acid Black 52 evidently are unknown.

In this study a substantial amount of time was devoted to seeking correlations between dye structure and toxicity to fish. Several observations appear to be appropriate: 1) None of the direct of vat dyes were toxic and most disperse dyes were not; 2) Mordant Black 11 and Acid Black 52, similar to each other in structure, had similar TL_{50} values (6 and 7 mg/l, respectively); 3) The triphenylmethane dyes were the most toxic of those tested (The triaminophenylmethane, Basic Violet 1, was toxic at a lower concentration than was the diaminophenylmethane, Basic Green 4); and 4) There appears to be a strong correlation between cationic ionization and toxicity.

Overall, it may be concluded that the cationic dyes are the most likely to be toxic. This is not surprising, as it has been known since the 1920's that many organic cations are effective antibacterial agents. Indeed, much of the early work on antibacterial activity of organic cations involved the triphenylmethane dyes and the aminoacridines (17).

In view of this conclusion, it should be stressed that pH may affect toxicity by influencing degree of ionization of the dye and degree of ionization of its site of action on the test organism. In studies performed for ADMI, in which only dye samples and clean water were used, the pH remained near neutrality. If, in actual practice, dyes were discharged in conjunction with acid or alkaline materials which would substantially change the pH, the toxic effect could be markedly changed. Commonly, however, dye waste streams receive primary and biological (secondary) treatment. As biological treatment demands a nearly neutral pH for effectiveness and since discharge of strongly acid or alkaline wastes to streams is not generally permitted, it would appear that marked variations in pH would not be expected.

SIGNIFICANCE OF STUDIES AND FURTHER RESEARCH GOALS

A 1936 "state-of-the art" survey of textile waste treatment described dye wastes as large in volume, high in color and, in many instances, toxic (7). Sulfur dyes and aniline black dyes were especially incriminated for their effects on sewage treatment plant and receiving stream biota. More than 25 years later another review of toxic effects of dyes concluded

> Despite the fact that dyes are used extensively in industry and despite the fact that numerous references state that dyes are toxic substances, relatively few specific references dealing with the toxicity of dyes were uncovered in this survey (22).

Today, nearly a decade since that review, the same statement may be made again, as evidenced in the review of Kemp, et al (4).

The research described in this report constitutes the first extensive study of effects of dyes on fish. The data indicate that many dyes should present no practical problem from the point of view of toxicity to fish because their threshold values are far above concentrations which could be acceptable in streams, considering regulatory limitations on color. On the other hand, data for others suggest the possibility of environmental problems relating to fish toxicity. These include dyes for which the observed TL_{50} concentrations, reduced by customary "application factors" to yield probable safe levels, result in concentrations below those which would cause objectionable color in streams. With these, it is possible that toxicity to fish could be a problem, even at concentrations lower than those deemed objectionable in the stream for reasons of appearance.

Wastewaters containing dyes may pass into the environment in practice via municipal sewerage systems or through direct discharge to streams by industries using them. In either event, pollution control regulations today require that virtually all wastewaters discharged by either route must be subjected to specified types of treatment before reaching the stream. In many instances, it is possible that the dye may be removed by a wastewater treatment process or modified to produce a less toxic form, such as by attachment of the dye to suspended materials removed during primary treatment or to growths developed in the biological system. For example, some of the dyes with relatively low TL_{50} values have been used as biological stains because of strong tendencies to combine with cell materials.

In view of this situation, it is logical to anticipate the toxicity of some of the dyes could be substantially less in practice than suggested by the rather simplistic tests conducted during these preliminary investigations. Further studies are underway of those dyes showing significant toxicity in the tests.

For those dyes for which the 96 hour TL_{50} to fathead minnows is <50 mg/l, aerobic biological treatment is under evaluation as a means of reducing color and toxicity.

For these tests, fill-and-draw activated sludge pilot plants with 10.5 gallon aeration capacity and 24 hour aeration time are employed. The units are seeded initially with mixed liquor from an extended aeration plant. Feed to the units is Chapel Hill sewage, to which sodium bicarbonate is added to increase alkalinity by 50-75 mg/l (as $CaCO_3$). For each test unit, the sewage is spiked with a test dye to give an initial dye concentration of 10-50 mg/l, depending on the TL_{50}. A control unit, identical to test units in every way except for dye addition, is operated concurrently with all test runs.

Aeration units are monitored daily for DO, pH, and temperature, and 5 times weekly for MLSS and MLVSS. Nitrogen forms (nitrite, nitrate, ammonia, and Kjeldahl) are measured 3 times per week.

After a minimum of 14 days of operation to insure development of an acclimated sludge, influent and effluents (2 day composites) are collected for analysis of BOD_5, TOC, nitrogen forms, and suspended solids. Additionally, effluents are evaluated for color (1) and fish toxicity.

Preliminary results indicate that secondary treatment generally achieves some reduction of color and toxicity, in some cases by 90-95%. However, this degree of treatment is inadequate with most of the dyes tested because the residual color and/or toxicity exceed acceptable levels. Therefore, investigations are being initiated to investigate physico-chemical methods of treatment (ozonation, coagulation-precipitation, activated carbon, ion-exchange) for reduction of color and toxicity.

. . . no matter what result is predicted,
the fish is the only true arbiter of toxicity (24)

APPENDIX

Dye Sample Preparation for ADMI Study

The samples of dyes provided by the ADMI for environmental studies are all composite samples from a variety of manufacturers. Following is a description of how the samples were prepared.

The list of dyes selected for evaluation was circulated to the member companies of ADMI with an invitation to provide as many as possible of the dyes on the list before a deadline date. The instructions provided the manufacturers asked that the Acid, Basic, Direct, Mordant Black 11 and Fluorescent Brightening Agent 28 samples be dried and ground concentrate with no additives present; the Disperse and Vat samples be finished, wet presscake with no additives present and that the Sulfur Black 1 be in solution with no additives present. The presscakes of dyes as produced in ordinary manufacture vary considerably with respect to moisture content and are filtered from solutions that contain amounts of salts, especially sodium chloride and sodium sulfate, that vary from almost none to saturated. For this reason the organic dye content of dried presscake will vary from about 100% to about 50% in the case of very wet pastes taken from saturated salt solutions.

The samples collected by the manufacturers were then sent to designated collecting points as follows:

1. Acid and Basic dyes — GAF
2. Direct, Mordant and Fl. Brightening — Verona
3. Vat and Sulfur dyes Sodyeco
4. Disperse dyes DuPont

Equal quantities from each of the collected samples of the Acid, Basic, Direct, Mordant and Fluorescent Brightening Agent were found and blended to provide the samples used for study.

Equal quantities of the vat dye pastes were mixed together and an amount of Tamol SN (dispersing agent) equal to the weight of the active ingredient of dye was added. The mixture was then milled to provide a reasonable dispersion to contain 15% active ingredient (dye) and 15% Tamol SN. This material was used to provide the samples for study.

The disperse dye samples were prepared in the same manner as the vat dye samples excepting that Reax 85 A was used for the dispersing agent.

Reax 85A is a lignin sulfonate and Tamol SN is a condensed naphthalene sulfonic acid. Samples of the same lot of materials used to make the dispersions for study have been retained.

Portions of the blended dye samples as provided for study have been retained for possible future use.

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INVESTIGATIONS IN FISH CONTROL

18. Toxicity of 22 Therapeutic Compounds to Six Fishes

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Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
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ABSTRACT.--Of 22 therapeutic chemicals (18 parasiticides and 4 oral bacteriostats) tested by bioassays, 16 were toxic to fish and 6 were not. Tests were in 24- and 48-hour static bioassays on rainbow, brown, brook, and lake trout and bluegills at 12° C. and channel catfish at 17° C. The 16 toxic chemicals, in descending order, were malachite green, Trolene, CoRal, Tiguvon, Roccal, P.M.A., Acriflavine, amopyroquin dihydrochloride, merthiolate, methylene blue, Neguvon, Ruelene, TV-1096, nickel sulfate, formalin, and quinacrine hydrochloride; the 6 that did not appear to be toxic were erythromycin thiocyanate, quinine hydrochloride, Flagyl, sulfamerazine, sulfamethazine, and sulfisoxazole.

An objective of the Fish Control Laboratories is to develop chemical tools to prevent and control fish diseases. Although efficacious concentrations of many drugs have been determined, a thorough examination of their toxicity has not been reported. Prior to clearance of drugs, the Food and Drug Administration requires data on their toxicity. The purpose of this study was to define the toxicity of 22 therapeutic chemicals to six species of fish before further research is undertaken on their efficacy and residues.

Eighteen parasiticides of known or possible value as external treatments for fish were selected for investigation upon recommendations by other investigators. Four oral bacteriostats were tested to determine whether any toxicity to fish would result through leaching, or excretion, of the compounds into water.

MATERIALS AND METHODS

Six species of fish were obtained from various fish hatcheries (table 1). All were quarantined for 10 days, and those judged acceptable for bioassays were acclimated to conditions of the tests.

The static bioassays were made in facilities described by Lennon and Walker (1964). We used 5-gallon glass jars which contained 15 liters of reconstituted, deionized water at total hardness of 42 p.p.m., and a maximum of 1 gram of fish per liter of water. Each test included 10 concentrations of a chemical. Ten fish were exposed to each concentration, and 20 fish served as controls.

The 22 therapeutic compounds were tested at 12° C. against five species of fish at La Crosse, Wis. (table 2). Tests against channel catfish at 17° were made at the Southeastern Fish Control Laboratory, Warm Springs, Ga.

A concentrated stock solution of each compound, using acetone or deionized water or both as solvents, was usually prepared for addition to the test vessels immediately before each test. When solubility of the compound prevented preparation of concentrated stocks, the compound was added directly and allowed to dissolve in the test vessel.

Observations on survival and mortality were recorded at 24 and 48 hours. The data were then analyzed by plotting concentration against mortality on logarithmic normal

TABLE 1.--Fishes used in toxicity trials

Species	Lot	Average length (inches)	Average weight (grams)	Grading date	Source
Rainbow trout, Salmo gairdneri.....	159	1.5	0.5	1-21-65	National Fish Hatchery, Manchester, Iowa
Do.....	159	1.8	0.9	2-15-65	
Brown trout, Salmo trutta.....	177	1.7	0.8	3-16-65	National Fish Hatchery, Lake Mills, Wis.
Do.....	177	1.9	1.2	4- 1-65	
Brook trout, Salvelinus fontinallis....	161	1.5	0.4	1-21-65	State Fish Hatchery, St. Croix Falls, Wis.
Do.....	161	1.6	0.6	2-15-65	
Lake trout, Salvelinus namaycush.....	78	4.0	2.5	8-14-64	National Fish Hatchery, Jordan River, Mich.
Do.....	78	4.0	2.8	8-28-64	
Do.....	78	4.1	3.2	10- 7-64	
Channel catfish, Ictalurus punctatus...	W-70	2.1	1.2	7-21-65	National Fish Hatchery, Marion, Ala.
Do.....	W-74	2.2	1.5	8- 4-65	
Bluegill, Lepomis macrochirus.....	115	1.6	0.8	11- 5-64	National Fish Hatchery, Lake Mills, Wis.
Do.....	131	1.4	0.7	11-17-64	
Do.....	131	1.7	1.1	12- 1-64	

TABLE 2.--Common names and active ingredients of compounds tested

Common name	Grade or formulation	Active ingredient
Acriflavine (neutral).....	technical.....	3,6-diamino-10-methyl acridinium chloride and 3,6-diaminoacridine
Amopyroquin dihydrochloride.....	technical.....	4-(7-chloro-4-quinolylamino)-α-1-pyrrolidyl-o-cresol dihydrochloride
CoRal.....	technical.....	O,O-diethyl O-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl-phosphorothioate
Erythromycin thiocyanate.....	800 mcg/mg.....	erythromycin thiocyanate
Flagyl.....	technical.....	1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole
Formalin.....	U.S.P.....	37-percent formaldehyde gas in water
Malachite green.....	technical.....	bis-(p-dimethylaminophenyl) phenylmethane treated with HCl
Merthiolate.....	technical.....	sodium ethylmercurithiosalicylate
Methylene blue.....	technical.....	3,7-bis(dimethylamino) phenazathionium chloride
Neguvon.....	80-percent soluble powder.....	O,O-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate
Nickel sulfate.....	analytical reagent.....	$NiSO_4 \cdot 6H_2O$
P.M.A.....	technical.....	pyridylmercuric acetate
Quinacrine hydrochloride (Atabrine).....	technical.....	3-chloro-7-methoxy-9- (1-methyl-4-diethylaminobutylamino) acridine dihydrochloride
Quinine hydrochloride.....	technical.....	quinine hydrochloride
Roccal.....	50-percent concentrate.....	alkyl dimethylbenzylammonium chloride
Ruelene.....	227 mg/cc.....	4-tert-butyl-2-chlorophenyl methyl methylphosphoramidate
Sulfamerazine.....	U.S.P.....	N^1-(4-methyl-2-pyrimidyl) sulfanilamide
Sulfamethazine.....	U.S.P.....	N^1-(4,6-dimethyl-2-pyrimidinyl) sulfanilamide
Sulfisoxazole.....	U.S.P.....	N^1-(3,4-dimethyl-5-isoxazolyl) sulfanilamide
Tiguvon.....	300 mg/cc.....	O,O-dimethyl O-[4-(methylthio)-m-tolyl] phosphorothioate
Trolene.....	technical.....	O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothioate
TV-1096 (Parke, Davis & Company).....	technical.....	1g-threo-2-(5-nitro-2-furyl)-5-(p-nitrophenyl)-2-oxazoline-4-methanol

(probability) graph paper to define the concentration that produced 50-percent mortality (LC_{50}) as described by Litchfield and Wilcoxon (1949). Variance and the 95-percent confidence interval (C.I.) were also determined.

Most of the compounds tested were technical or U.S.P. materials, and the rest were formulated materials. To eliminate confusion, all results are reported in terms of p.p.m. of total material (formulated or technical) instead of active ingredient.

RESULTS

Of the 22 compounds, 16 were toxic to the six species of fish, and the LC_{50} values were determined (tables 3 to 8).

The most toxic compound, malachite green, is relatively uniform in toxicity to the six

TABLE 3.--Toxicity of 16 compounds to rainbow trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	30.1	26.2-34.6	19.9	17.0-23.3
Amopyroquin	47.0	43.5-50.8	35.3	33.3-37.4
CoRal	2.60	2.28-2.96	0.55	0.51-0.59
Formalin	207	182-236	168	154-183
Malachite green	0.50	0.36-0.69	0.39	0.33-0.46
Merthiolate	60.5	53.5-68.4	21.2	18.6-24.2
Methylene blue	25.0	20.5-30.5	16.0	13.8-18.7
Neguvon	32.5	29.3-36.1	12.2	10.6-14.0
Nickel sulfate	320	302-339	160	150-171
P.M.A.	5.00	4.35-5.75	3.75	3.32-4.65
Quinacrine HCL	--	--	172	159-186
Roccal	3.24	2.92-3.60	2.57	2.16-3.06
Ruelene	35.0	31.5-38.8	32.0	30.5-33.6
Tiguvon	5.30	4.82-5.83	4.35	3.62-5.22
Trolene	1.17	0.89-1.53	0.74	0.64-0.86
TV-1096	24.2	22.8-25.7	16.1	14.9-17.4

TABLE 4.--Toxicity of 16 compounds to brown trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	40.0	36.4-44.0	27.0	25.0-29.2
Amopyroquin	42.0	37.5-47.0	36.0	33.3-38.9
CoRal	0.92	0.84-1.00	0.73	0.62-0.86
Formalin	325	304-348	185	165-208
Malachite green	0.45	0.42-0.49	0.34	0.30-0.38
Merthiolate	110	75-160	54.0	47.8-61.0
Methylene blue	54.0	46.2-63.2	32.8	28.3-37.4
Neguvon	54.0	48.2-60.5	16.5	11.3-23.1
Nickel sulfate	400	345-464	270	241-302
P.M.A.	9.30	8.30-10.42	6.22	5.71-6.78
Quinacrine HCL	390	361-421	230	184-288
Roccal	2.95	2.46-3.54	2.05	1.74-2.42
Ruelene	26.2	24.7-27.8	25.7	24.2-27.2
Tiguvon	4.90	4.09-4.95	3.62	2.78-4.71
Trolene	0.53	0.38-0.74	0.39	0.30-0.51
TV-1096	--	--	--	--

TABLE 5.--Toxicity of 16 compounds to brook trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	48.0	43.2-53.3	14.8	14.0-15.7
Amopyroquin	52.0	44.8-60.3	40.0	38.1-42.0
CoRal	1.06	0.87-1.29	0.80	0.70-0.91
Formalin	196	187-206	157	143-173
Malachite green	0.30	0.22-0.40	0.26	0.22-0.31
Merthiolate	89.5	85.2-94.0	74.5	71.0-78.2
Methylene blue	49.8	41.2-60.3	22.9	17.2-30.5
Neguvon	14.0	23.4-49.3	16.8	14.1-20.0
Nickel sulfate	--	--	242	224-261
P.M.A.	15.5	12.9-18.6	10.7	9.8-11.7
Quinacrine HCL	--	--	230	177-299
Roccal	4.13	3.79-4.50	3.40	3.09-3.74
Ruelene	36.8	34.4-39.4	35.0	31.5-38.8
Tiguvon	6.15	5.21-7.26	5.50	5.14-5.88
Trolene	0.59	0.44-0.78	0.39	0.26-0.59
TV-1096	29.1	26.4-32.5	19.0	16.8-21.5

TABLE 6.--Toxicity of 16 compounds to lake trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	37.5	34.7-40.5	28.0	24.4-32.2
Amopyroquin	15.5	12.1-19.8	14.0	10.8-18.1
CoRal	6.80	4.00-11.56	4.00	1.25-12.80
Formalin	220	200-242	167	160-174
Malachite green	0.57	0.49-0.66	0.40	0.33-0.49
Merthiolate	13.0	9.6-17.6	2.13	1.06-4.26
Methylene blue	35.0	29.4-41.6	34.0	29.3-39.4
Neguvon	41.0	38.7-46.5	9.00	7.20-11.25
Nickel sulfate	170	139-209	75.0	55.6-101.2
P.M.A.	12.5	11.8-13.2	7.60	6.33-9.12
Quinacrine HCL	28.0	18.8-42.0	21.0	12.4-35.7
Roccal	2.70	2.41-3.02	1.95	1.68-2.26
Ruelene	27.0	25.0-29.2	27.0	23.9-30.5
Tiguvon	6.50	6.08-6.96	5.30	4.91-5.72
Trolene	0.73	0.62-0.86	0.62	0.53-0.72
TV-1096	32.0	28.6-35.8	16.5	13.2-20.6

TABLE 7.--Toxicity of 16 compounds to channel catfish at 17° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	43.5	39.9-47.4	33.2	31.0-35.5
Amopyroquin	19.8	17.7-22.2	12.5	11.3-13.2
CoRal	6.80	5.81-7.96	--	--
Formalin	137	129-145	96.0	90.6-101.8
Malachite green	0.21	0.17-0.27	0.20	0.16-0.26
Merthiolate	7.50	6.41-8.78	5.65	4.79-6.67
Methylene blue	120	110-131	104	93-116
Neguvon	80.0	72.7-88.0	32.0	24.8-41.3
Nickel sulfate	368	334-405	165	129-211
P.M.A.	3.22	2.86-3.90	2.89	2.60-3.21
Quinacrine HCL	198	169-232	70.0	59.3-82.6
Roccal	1.28	1.16-1.41	1.12	1.03-1.22
Ruelene	39.5	37.6-41.5	34.8	32.5-37.2
Tiguvon	5.90	4.50-7.73	5.30	4.50-7.73
Trolene	1.76	1.54-2.01	1.26	1.09-1.46
TV-1096	27.0	24.8-29.4	20.3	19.3-21.3

TABLE 8.--Toxicity of 16 compounds to bluegills at 12° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	18.0	16.8-19.3	13.5	12.6-14.4
Amopyroquin	33.0	23.6-42.2	18.5	16.7-20.5
CoRal	10.5	8.1-13.6	8.00	6.11-10.48
Formalin	185	156-220	140	127-154
Malachite green	0.26	0.22-0.31	0.11	0.09-0.14
Merthiolate	110	87-139	64.5	57.6-72.2
Methylene blue	51.0	40.2-64.8	33.0	26.2-41.6
Neguvon	78.0	64.5-94.4	71.0	55.9-90.2
Nickel sulfate	--	--	495	450-544
P.M.A.	20.0	18.0-22.2	16.0	13.4-19.0
Quinacrine HCL	120	73-198	79.0	54.1-115.3
Roccal	2.10	1.84-2.27	1.68	1.56-1.81
Ruelene	16.0	14.1-17.8	15.0	13.0-17.1
Tiguvon	15.7	13.2-18.7	8.90	7.67-10.32
Trolene	2.50	2.25-2.78	1.30	0.67-1.50
TV-1096	37.0	33.3-41.1	28.2	25.9-30.7

species, and LC_{50} values range from 0.11 to 0.40 p.p.m. at 48 hours. Clemens and Sneed (1958a) reported its LC_{50} to channel catfish as 0.14 p.p.m. in 24 and 48 hours at 25° C. Our results show the LC_{50} values to be 0.21 and 0.20 p.p.m. in 24 and 48 hours respectively at 17°. This variation between results may be due to differences in test temperatures.

Following malachite green in decreasing order of toxicity are Trolene, CoRal, and Tiguvon, all of which have the basic structure of phosphorothioate. In the same general range of toxicity are Roccal and P.M.A., with Roccal the more toxic of the two. Roccal, like malachite green, exhibits relatively uniform toxicity, and LC_{50} values range from 1.12 to 3.40 p.p.m. at 48 hours for all species.

P.M.A. exhibits a much wider range of toxicity with LC_{50} values of 2.9 to 16.0 p.p.m. at 48 hours. Clemens and Sneed (1958a) reported its LC_{50} to channel catfish as 3.8 p.p.m. in 24 hours at 24° C. In a later publication, these authors (1958b) reported the LC_{50} of P.M.A. to channel catfish as 2.96 and 2.81 p.p.m. in 24 and 48 hours, respectively, at 16.5°. Both reports compare favorably with our LC_{50} values of 3.22 and 2.89 p.p.m. for 24 and 48 hours at 17°.

Acriflavine, amopyroquin dihydrochloride, merthiolate, methylene blue, Neguvon, Ruelene, and TV-1096 fall into an intermediate toxicity range with LC_{50} of 10 to 100 p.p.m. Only Ruelene exhibits a uniform LC_{50} range of 25.7 to 35.0 p.p.m. for six species in 48 hours. The other compounds of this group demonstrate a relatively wide range of toxicity to the different species.

TV-1096 is soluble only to approximately 30 p.p.m. in water. Amounts above this level produce a saturated solution with a precipitate on the bottom of the test vessel. Brown trout fail to succumb to concentrations below 30 p.p.m. and for this reason, LC_{50} values could not be derived for the species. Also, amounts of TV-1096 in excess of a saturated solution are nontoxic to brown trout. In contrast, LC_{50} values of TV-1096 for the other species range from 16.1 to 28.2 p.p.m. at 48 hours.

Nickel sulfate, formalin, and quinacrine hydrochloride are the least toxic of the compounds analyzed. Formalin exhibits a fairly uniform LC_{50} range of 96 to 185 p.p.m. at 48 hours. The other two have a much wider range.

Clemens and Sneed (1958a) reported the LC_{50} values of formalin on channel catfish to be 87 and 69 p.p.m. in 24 and 48 hours, respectively, at 25° whereas we found them to be 137 and 96 p.p.m. in 24 and 48 hours, respectively, at 17° C. This variation in results seems to indicate that the toxicity of formalin may be increased by an increase in temperature. The observation is supported by our results which show that formalin is more toxic to channel catfish at 17° than it is to four species of trout and to bluegills at 12°.

Erythromycin thiocyanate and quinine hydrochloride were tested at an arbitrary level of 100 p.p.m. Their solubility would have permitted higher concentrations but preliminary tests indicated little toxicity. At 100 p.p.m., the substances were not toxic to the fish.

The poor solubility of Flagyl, sulfamerazine, sulfamethazine, and sulfisoxazole prevented the determination of LC_{50} values. Solutions were saturated before lethal levels could be reached. The arbitrary concentration of 100 p.p.m. was selected then for tests. This resulted in saturated solutions with excess chemical remaining on the bottom of the bioassay vessels. None of them was toxic to the six species of fish.

DISCUSSION

Malachite green has been in use for many years as a fungicidal dip for fish (Foster and Woodbury, 1936). Recently, Amlacher (1961) recommended it for prolonged treatment of fish in ponds to combat Ichthyophthirius, Chilodenella, and Costia. He applied 0.15 p.p.m. and allowed it to dissipate in the water. Concentrations of 0.11 to 0.40 p.p.m. in our bioassays proved toxic within 48 hours to the six species of fish tested. Thus, there is a risk with concentrations over 0.11 p.p.m. in long-term treatments.

Trolene, CoRal, and Tiguvon are under consideration as prolonged treatments for control of Ichthyophthirius. Tiguvon is the least toxic of these organophosphates to fish. This indicates that it may prove the most valuable of the group if minimum concentrations required for control of "Ich" are approximately the same for all three.

Roccal has been in use as a bactericide for many years (Fish, 1947). Putz (1964) reported its possible value in prolonged, indefinite treatments at 0.25 to 0.50 p.p.m. for Ichthyophthirius. In treatments such as this, the chemical attacks the free-living stages of "Ich". He did not say which formulation of the chemical he used, but 10-percent active is the formulation commonly used in hatcheries (Davis, 1956). We used 50-percent active, and upon converting from 10-percent active to 50-percent active, the treatment levels could be reduced to 0.05 and 0.10 p.p.m. This permits a comparison between treatment levels and toxicity which shows a 10-fold difference in concentrations.

P.M.A. has been of considerable value in combating bacterial and protozoan diseases (Davis, 1956). Evidence of its greater toxicity to rainbow trout than other trouts has been reported over the years (Foster and Olson, 1951; Rodgers et al., 1951; Wolf, 1951; Hammer, 1960). Allison (1957) reported variations in the toxicity of P.M.A. from lot to lot of chemical. We used only one lot of P.M.A. in this study, and the results support the earlier findings that it has greater toxicity to rainbow trout. For example, it was up to three times as toxic to rainbow trout as to brook trout. Channel catfish appear to be sensitive to the compound at 17° C.

Snieszko and Friddle (1948) used merthiolate (sulfo) as a disinfectant for rainbow trout eggs. Van Duijn (1956) cautioned against use of merthiolate as a fish bath since the compound is a mercurial and is certain to be toxic to fish in contact with it for some period. We find an extreme variation in its toxicity to different species. This is especially true at 24 hours where LC_{50} values range from 7.5 p.p.m. for channel catfish to 110.0 p.p.m. for brown trout. This variation diminishes somewhat at 48 hours, and lake trout become the most sensitive to the chemical. The LC_{50} at 48 hours for lake trout is 2.1 p.p.m. in contrast with 74.5 p.p.m. for brown trout. Variations in resistance such as this may make merthiolate extremely difficult to work with in routine treatments of several species.

Acriflavine, amopyroquin dihydrochloride, methylene blue, Neguvon, Ruelene, and TV-1096 are under consideration as prolonged, indefinite treatments for control of Ichthyophthirius. Putz (1964) reported that 3 p.p.m. of acriflavine shows promise against the parasite. Our results indicate that bluegills are the most sensitive to the compound with LC_{50} values of 18.0 and 13.5 p.p.m. at 24 and 48 hours respectively. Channel catfish are the most resistant with LC_{50} values of 43.5 and 33.2 p.p.m. at 24 and 48 hours.

Clemens and Sneed (1958a) reported the LC_{50} values of acriflavine on channel catfish at 24 and 48 hours to be 11.5 and 6.8 p.p.m., respectively, at 20° C. Our finding, is that it is only about one-fourth as toxic as that. Possible causes for the discrepancy are many. Among them are differences in water quality and temperature, differences in the condition of fish, and purity of the compound used. In addition to this unexplained variation in the toxicity of acriflavine, another factor warrants serious consideration in its use. Van Duijn (1956) reported sterility in both egg-laying and live-bearing aquarium fish. This is a temporary situation and normal fertility is restored after several months.

Amopyroquin dihydrochloride also shows promise as a prolonged, indefinite treatment at 0.05 to 0.10 p.p.m. for control of Ichthyophthirius (Putz, 1964). Our results show that its toxicity to all trout tested, with the exception of lake trout, is between 35 and 40 p.p.m. for 48 hours. Also, bluegills at 12° C. and channel catfish at 17° are approximately as sensitive as lake trout. A treatment level of 0.1 p.p.m. would include a safety margin in use of more than a hundredfold even against these three species.

Van Duijn (1956) recommended methylene blue as a satisfactory control for Ichthyophthirius in aquariums. He used 2 to 4 p.p.m.

of it in a permanent bath at temperatures between 21 and 27° C. We found that rainbow trout are the most sensitive to the dye, and the LC_{50} is 16 p.p.m. at 48 hours. The most resistant species is channel catfish with an LC_{50} of 104 p.p.m. at 48 hours. The remaining species are intermediate in sensitivity with a 48-hour LC_{50} range of 22.9 to 34.0 p.p.m. Comparison of the use levels with toxicity levels indicates a good safety margin.

Neguvon and Ruelene are of approximately the same toxicity except in one very important respect. Neguvon has a marked difference between the 24-hour and 48-hour LC_{50}. The most striking example of this involves lake trout with LC_{50} values of 41 p.p.m. at 24 hours and 9 p.p.m. at 48 hours. Differences between the 24- and 48-hour LC_{50} values by a factor of at least two are common except with bluegills. For some unknown reason the difference with bluegills is only 78 to 71 p.p.m.

Ruelene provides a contrast with Neguvon because it exhibits approximately the same toxicity at 24 and 48 hours. In the case of lake trout, the 24- and 48-hour LC_{50} values are identical at 27 p.p.m. It is possible that Ruelene degrades very rapidly in the test vessel to a nontoxic level.

TV-1096 has toxicity comparable to that of Neguvon and Ruelene. Like Neguvon, it does not appear to degrade as rapidly as Ruelene.

Nickel sulfate is under consideration as a prolonged, indefinite treatment for control of Ichthyophthirius. Our results show that it is relatively low in toxicity when compared with the other compounds tested, but it has a fairly wide range of toxicity among the species tested. The LC_{50} values at 48 hours range from 75 p.p.m. for lake trout to 495 p.p.m. for bluegills. Twenty-four-hour tests of 50 to 275 p.p.m. on brook trout and 200 to 500 p.p.m. on bluegills did not cause death.

Allison (1957) reported use of formalin as a parasiticide in long-term treatments in ponds. He suggested 5 p.p.m. for Gyrodactylus and 15 p.p.m. for Trichodina and Ichthyophthirius. Our results show that formalin is relatively and uniformly low in toxicity when compared with the other compounds tested. It does appear to increase in toxicity as temperatures rise from 17° to 25° C. Even with this increase in toxicity, the compound retains a safety margin of at least sixfold at recommended use levels.

Van Duijn (1956) recommended use of quinacrine hydrochloride in treatment of stubborn cases of "Ich" in aquarium fish. The treatment consists of three applications of 1 p.p.m. at 48-hour intervals. This totals 3 p.p.m. if no degradation of the compound occurs. He also stated that this treatment should not be extended over long periods and that 8 to 10 days should be sufficient.

Our results show that lake trout are approximately 10 times as sensitive to quinacrine hydrochloride as the other trout and 3 or 4 times as sensitive as bluegills and channel catfish. The sensitivity is complicated by the fact that the toxicity to lake trout is quite erratic and some deaths occur over a wide range of concentrations. The 48-hour LC_0 of quinacrine hydrochloride for lake trout is approximately 10 p.p.m., the LC_{50} is 21 p.p.m., and the LC_{100} is 110 p.p.m. Some fish succumb to the chemical quickly and at comparatively low concentrations whereas the rest survive for long periods. Further evidence of this lingering is shown by the slight difference between the 24-hour LC_{50} of 28 p.p.m. and the 48-hour LC_{50} of 21 p.p.m. In contrast, there is a considerable difference between the 24- and 48-hour LC_{50} values obtained for the other species. A possible explanation is that there is considerable variation in resistance among lake trout individuals.

Van Duijn (1956) recommended use of quinine hydrochloride for treatment of Ichthyophthirius in aquarium fish. The treatment consists in adding 1 p.p.m. on 3 successive days, a final treatment level of 3 p.p.m. He cautioned against use of the treatment for long periods because of possible fertility problems. Our results show that 100 p.p.m. of the chemical in water are not toxic within 48 hours to the species tested.

Erythromycin thiocyanate has been used as a food additive for control of kidney disease in rainbow trout at 4.5 grams per 100 pounds of

fish per day for 21 days (Piper, 1961). Warren (1963) reported that it is toxic to rainbow trout at 500 mg. per kg. Our results show that 100 p.p.m. of the antibiotic in water is not toxic to the species tested within 48 hours.

Flagyl has been used in medicine as an antiprotozoal agent (Cutting, 1962). Putz (1964) reported experimental use of it at 1.5 p.p.m. for control of Ichthyophthirius. Our results show that Flagyl is nontoxic at 100 p.p.m. The finding is qualified somewhat since the compound is not immediately soluble at 100 p.p.m. It dissolves slowly, however, and is completely in solution within 48 hours.

Snieszko and Bullock (1957) reported use of sulfamerazine, sulfamethazine, and sulfisoxazole as food additives in the treatment of furunculosis at 8 to 10 grams per 100 pounds of fish per day for 10 to 20 days. Van Duijn (1956) recommended use of the sodium salt of sulfamerazine at 1 part per thousand as an effective cure for worm cataract in aquarium fish. In our water, sulfamerazine, sulfamethazine, and sulfisoxazole are not soluble at 100 p.p.m., and saturated solutions are not toxic to the six species within 48 hours.

All of our results were obtained with fish which were, to the best of our knowledge, healthy. They showed no signs of disease or physical injuries. The toxicity of these compounds to fish which are sick or in poor condition might be significantly different.

None of the compounds reported herein are cleared by the Food and Drug Administration and the Department of Agriculture for use on fish destined for human consumption. The data and discussion presented in this paper should not be construed as recommendations for use.

CONCLUSIONS AND SUMMARY

The toxicities of 22 therapeutic compounds to rainbow trout, brown trout, brook trout, lake trout, and bluegills at 12° C. and channel catfish at 17° were determined in 24- and 48-hour static bioassays.

LC_{50} values for malachite green, the most toxic compound tested, range from 0.1 to 0.4 p.p.m. for all species tested. CoRal, P.M.A., Roccal, Tiguvon, and Trolene are less toxic than malachite green, but still rank relatively high in toxicity. Their LC_{50} values range from approximately 0.5 to 10 p.p.m. for all species.

Acriflavine, amopyroquin dihydrochloride, merthiolate, methylene blue, Neguvon, Ruelene, and TV-1096 are intermediate in toxicity. The LC_{50} values range from approximately 10 to 100 p.p.m. for all species. Merthiolate has wide variations in toxicity to various species.

Formalin, nickel sulfate, and quinacrine hydrochloride have relatively low toxicities. The LC_{50} values are usually above 100 p.p.m. Quinacrine hydrochloride is substantially more toxic to lake trout than to the other species.

No tests of erythromycin thiocyanate and quinine hydrochloride were made at concentrations above 100 p.p.m. They are not toxic within 48 hours at this concentration. Saturated solutions of Flagyl, sulfamerazine, sulfamethazine, and sulfisoxazole are also not toxic within 48 hours.

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